Progeny Quality of *Gonatocerus ashmeadi* (Hymenoptera: Mymaridae) Reared on Stored Eggs of *Homalodisca coagulata* (Hemiptera: Cicadellidae)

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ABSTRACT This study assessed the effects of refrigerated storage on the suitability of eggs of the glassy-winged sharpshooter, *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae), as hosts for propagation of the parasitoid *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae). Development of the host eggs was terminated by chilling at 2°C for 5 d before storage was initiated at 10°C for up to 70 d. Parasitism, adult emergence rate, developmental time, and sex ratio were used to gauge the suitability of the eggs as hosts after storage. In addition to these measures, demographic growth parameters also were used to assess the quality of the wasp progeny through the F_2 generation. Host eggs stored 20 d remained fully acceptable to the wasps for attack. Although the parasitism rate decreased with storage time, > 80% adult parasitoid emergence was realized from eggs stored 30 d. After 70 d storage, adult emergence rate was decreased by 48%, fecundity decreased by 53%, female production by 19%, developmental time was extended 3 d, and female longevity was shortened 5 d. The emergence pattern of F_1 but not F_2 adults varied with storage time of the parental and grandparental hosts, respectively. For the F_2 generation, emergence rate, development, and sex ratio did not vary with storage time when the F_1 parents parasitized fresh host eggs. Demographic parameters for the F_1 population showed that net reproductive rate was > 20 although it decreased significantly after their parental host eggs were stored for > 30 d. The intrinsic and finite rates of increase, population doubling time, and mean generation time decreased only after storage for 60 d. Our results show that short-term cold storage could be used for maintaining wasp populations in a mass-rearing program and that the detrimental effects of chilling host eggs in storage for over 30 d do not extend to F_2 generation.

KEY WORDS cold storage, glassy-winged sharpshooter, host suitability, fitness, egg parasitoid

The egg parasitoid, *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae), is one of the most common natural enemies associated with the glassy-winged sharpshooter, *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae), in California, the southeastern United States, and northeastern Mexico (Triapitsyn and Phillips 2000, Irvin and Hoddle 2005). *H. coagulata* is an efficient vector of the xylem-inhabiting bacterial pathogen *Xylella fastidiosa* Wells (Sorensen and Gill 1996), which causes a variety of scorch-like diseases in a range of high-value agricultural crops and ornamental plants (Purcell et al. 1999, Almeida and Purcell 2003). *G. ashmeadi* became associated with *H. coagulata* in California around 1990 (Sorensen and Gill 1996), and it accounts for 80–95% of the observed parasitism of sharpshooter eggs in California (Triapitsyn et al. 1998). Along with two other mymarid parasitoid species, *G. triguttattus* Girault and *G. fasciatus* Girault, *G. ashmeadi* has been released across nine California counties, and the impact of this release on controlling *H. coagulata* populations is being evaluated (CDFA 2003).

Classical biological control typically relies on techniques of mass production of natural enemies to amass large quantities of individuals for eventual release and establishment of field population (Miller 1995). In the absence of an effective artificial diet, the propagation of *G. ashmeadi* is currently dependent on sustainable supply of host eggs. During the prime reproductive season, *H. coagulata* eggs may be overproduced and discarded when demands for parasitoid releases are low. *H. coagulata* often has lower egg production when conditions for oviposition are unsuitable or a naturally occurring reproductive diapause is initiated (Lauzière et al. 2002, Hummel et al. 2006). Thus, a shortage of host eggs has a negative impact on parasitoid buildup in the field in the early spring and also on the maintenance of laboratory *G. ashmeadi* colonies.

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Low temperature storage is a strategy often used for obtaining efficiency and flexibility in the mass rearing of insects for release in biological control and sterile insect technique programs (Leopold 1998). It has been well documented that eggs can be stored at low temperatures for use as hosts for rearing parasitoids (Popov 1974, Fedde et al. 1979, Drooz and Solomon 1980, Drooz and Weems 1982, Correa-Ferreira and Moscardi 1993, Kivan and Kilic 2005). Preserving the host material in a state suitable for successful development of beneficial insect parasites or predators is especially important for the mass rearing of natural enemies. Accordingly, parasitism by the parasitoids and successful development of their progeny has been often used as the criteria to evaluate the suitability of cold-stored hosts for rearing parasitoids (Fedde et al. 1979, Drooz and Solomon 1980, Drooz and Weems 1982, Kivan and Kilic 2005).

Significant progress was made in a recent study that showed that *G. ashmeadi* successfully parasitized dead *H. coagulata* eggs that had also experienced short-term cold storage (Leopold and Chen 2005). The quality of the progeny of *G. ashmeadi* reared by this means was not yet examined. Quality assessment of parasitoids or hosts that have experienced cold storage is essential because there are numerous examples of detrimental effects on survival, development, and/or reproduction in cases where storage may have been too long or the temperature too low (Leopold 1998). Understanding of how low temperature storage of *H. coagulata* eggs affects the progeny fitness of *G. ashmeadi* will allow the design of a protocol for cold storage of *H. coagulata* eggs where the limits are defined and can be used in mass rearing of this parasitoid for future biological control programs. Thus, the purpose of the study presented here was to 1) determine the suitability of chill-terminated *H. coagulata* eggs exposed to cold storage for up to 70 d by examining the parasitism and development of *G. ashmeadi;* 2) determine the fitness of parasitoid progeny by examining the reproductive and developmental biology; and 3) assess the progeny quality derived from *G. ashmeadi* reared on refrigerated host eggs by using laboratory-generated demographical growth parameters.

Materials and Methods

Insect Rearing. The glassy-winged sharpshooter colony used in this study was initiated from insects acquired in 2001 from a laboratory colony maintained at the USDA–APHIS Plant Protection Laboratory, Edinburg, TX, which was originally collected near Bakersfield, CA. Our *G. ashmeadi* colony was started in 2003 from the colony maintained at the Mt. Rubidoux Field Station of the California Department of Food and Agriculture, Riverside, CA. Both the host and the parasitoid colonies were maintained in the laboratory by using the procedures described by Chen et al. (2006a).

Parasitism of Refrigerated Host Eggs. The glassy-winged sharpshooter colony was maintained within tent-like cages (30 by 30 by 30 cm, Bug Dorm-2, BioQuip Products, Rancho Dominguez, CA) that contained a mixed plant host system consisting of sunflower, *Helianthus annuus* L.; an evergreen shrub, *Euonymus japonica* Thunb.; and chrysanthemum, *Chrysanthemum morifolium* L. 'White Diamond' in a greenhouse augmented with high-intensity sodium lighting under a photoperiod of 16:8 (L:D) h and a temperature minimum of 27°C. The glassy-winged sharpshooter females typically deposit their eggs in groups on the underside of plant leaves beneath the leaf epidermis. The egg masses (< 24 h old) for this study were collected daily from only the *Euonymus* plants. The leaf bearing the egg mass and several other leaves with the shoot were removed from the plant. These cuttings bearing the egg masses were then placed vertically into plastic containers filled with water. Before initiating the storage experiments, all the cuttings were refrigerated at 2°C for 5 d to terminate development of the *H. coagulata* eggs (Leopold and Chen 2005). For this study, the 5-d treatment period was not calculated as part of the storage period. Thus, 5 d could be added to all storage times because they were, in fact, refrigerated and in the process of being preserved.

To determine the effect of refrigerated storage on the suitability of the chill-terminated eggs for parasitism by *G. ashmeadi*, egg masses were stored at 10°C under a photoperiod of 8:16 (L:D) for up to 70 d. Newly collected, untreated host eggs (<24 h old) were referred to as the control. At least 10 egg masses were removed at 10-d intervals and then exposed for 2 d to a *G. ashmeadi* colony, which contained ≈100 parasitoids (0–3 d old) in each tent-cage at 22 ± 1°C and photoperiod of 16:8 (L:D). Under these conditions, the ratio of females-to-males in this parasitoid colony is between 3.4 and 3.9 (Chen et al. 2006b). After exposure to the parasitoids, the host egg masses were incubated at 28 ± 2°C under continuous lighting (24:0 [L:D]) in a tent-cage until parasitoid emergence was detected. Developmental times of both sexes of the F_1 generation from oviposition to adult emergence were recorded. Three days after emergence ended, the egg masses were dissected under a stereoscope, and the number of emerged parasitoids was recorded. The host eggs having no parasitoid emergence were dissected and stained using the procedures described by Chen et al. (2006b) and the number of host eggs having dead parasitoid eggs or larvae or pupae also was recorded. Therefore, the total number of host eggs parasitized = the number of host eggs having emerged parasitoids + the host eggs containing dead parasitoid eggs, larvae, or pupae. Successful parasitism was estimated by using the ratio of the total number of host eggs parasitized compared with the number of test host eggs. This experiment was repeated five times.

Quality of F_1 Generation. To determine the effects of the storage duration of refrigerated host eggs on the quality of the F_1 generation, the parasitism, fecundity, and longevity of F_1 females and their progeny emergence, development, and sex ratio were examined. The treatment protocols included eight groups of parasitoid progeny collected from the experiments

described above and targeted 80 *H. coagulata* eggs (< 24 h old) per parasitoid female. Under this host/parasitoid ratio, superparasitism by this parasitoid was not observed (Chen et al. 2006a). To prevent egg and leaf desiccation, the petioles of excised *Euonymus* leaves, bearing a total of 80 host eggs, were placed into a moistened sponge in a petri dish (3.5 cm in diameter by 1.0 cm in height) within a transparent container (26 cm in diameter by 9 cm in height, Tri-state Plastic, Dixon, KY). The transparent containers, equipped with lids covered with fine nylon mesh, were placed upside down before inserting the petri dish. Because the number of eggs per egg mass was variable, accumulation of each of the samples of 80 host eggs used in this study consisted of six egg masses located on six leaves to avoid a possible effect of spatial heterogeneity on parasitoid behavior. Then, a mated female parasitoid was selected from pooled *G. ashmeadi* individuals (sex ratio was 1:1). These individuals were collected from the colony reared from refrigerated host eggs, and after emergence; they were caged with the males for 4–6 h while random mating occurred. Thus, one mated female was introduced into each container, and all experiments were conducted in a laboratory at 28 ± 2°C under continuous fluorescent lighting. After 24 h, the wasp in each container was removed and the survival was recorded. If a female died during experiment, data from that container were not used. After the removal of the parasitoids, the containers were placed at 28 ± 2°C under continuous fluorescent lighting to allow the progeny to develop into adults and emerge from the host eggs. During the experimental period water was provided via a wet sponge.

When parasitoid or *H. coagulata* emergence was first detected, the leaves bearing the egg masses were placed into a new container and checked daily for continued emergence. We recorded the developmental time of parasitoids from oviposition to emergence and the number of emerged female and male offspring produced by each wasp. Three days after emergence ceased, host egg masses were dissected under a microscope to record the number of emerged parasitoids and the number of parasitoid eggs, larvae, and pupae that died during development. Identification of *G. ashmeadi* immature stages and the dissection method were as described by Chen et al. (2006a). These experiments were replicated 10 times.

To determine longevity and fecundity of the F_1 generation, one mated female (≈4 h old) was introduced into the 26 cm diameter × 9 cm high container with 80 *H. coagulata* eggs (< 24 h old) on six excised *Euonymus* leaves. All the experiments were conducted in the laboratory (28 ± 2°C), with continuous lighting provided by fluorescent lights. After 24 h, females were moved to a new cage containing 80 more eggs of the same age and distribution. This procedure was repeated every 24 h until the parasitoid died. Water was provided via a moistened sponge. All the egg masses were dissected and the number of parasitoid eggs produced by each wasp was recorded. The experiment was repeated at least 10 times.

Calculation of Demographic Growth Parameters. Developmental time from oviposition to emergence, adult parasitoid survivorship, daily fecundity, and sex ratio of the progeny from *G. ashmeadi* reared from stored refrigerated host eggs were used to construct $l_x m_x$ life tables from which demographic growth parameters were calculated. Data from adult parasitoids dying shortly after handling were not used to calculate demographic parameters (storage time 0 d, $n = 14$; 10 d, $n = 10$; 20 d, $n = 10$; 30 d, $n = 13$; 40 d, $n = 15$; 50 d, $n = 20$; 60 d, $n = 12$; and 70 d, $n = 12$). The following parameters were estimated using methods as described by Carey (1993) to construct the $l_x m_x$ life table: intrinsic rate of increase, $r_m = (\ln R_0)/T$; net reproduction rate, $R_0 = \sum l_x m_x$; mean generation time, $T_c = \sum l_x m_x x)/\sum (l_x m_x)$; finite rate of increase, $\lambda = \exp(r_m)$; and doubling time, $T_d = \ln(2)/r_m$ (where l_x is the proportion of individuals alive at age x, and m_x is the number of female offspring produced per female during age interval x).

The jackknife technique was used to estimate mean demographic parameters of $l_x m_x$ for the life table and their SEs. This method was first applied to life table analysis as proposed by Meyer et al. (1986) and has been widely used to estimate population growth rates of animals in the past two decades. The jackknife analysis method removes one observation at a time from the original data set and recalculates the statistic of interest from the truncated data set. The method can estimate R_0, T_c, r_m, λ, and T_d, with their respective jackknife variances and confidence intervals. A Student's *t*-test was used to perform pairwise comparisons (two-tailed) (Maia et al. 2000) to determine whether the length of storage of the host of the parent had significant effects on the *G. ashmeadi* population growth statistics.

Statistical Analysis. Mean developmental time, parasitism, and emergence percentage, mean adult longevity, sex ratio, and daily and lifetime fecundity for the parasitoid were compared using one-way analysis of variance (ANOVA) (PROC ANOVA) and Tukey's studentized range test ($P = 0.05$) across all storage times of the refrigerated *H. coagulata* eggs. Two-way ANOVA (PROC GLM) was used to determine whether the interaction between sex and storage duration significantly affected by the developmental time of the F_1 and F_2 generations. Before analysis, data on parasitism, emergence, and mortality rates and percentage of male progeny were arcsine-transformed to meet the assumption of normality. All the statistical analysis was conducted by using the SAS program (SAS Institute 1996).

Results

Microscopic Examination of Refrigerated Host Eggs. Eggs of *H. coagulata* stored at 10°C for up to 70 d after development had been terminated by exposure to 2°C for 5 d exhibited four morphological states. Eggs, stored for 0–30 d (Fig. 1A) mostly seemed to be in the same condition as those that had been recently oviposited. They were sausage-shaped, arranged in

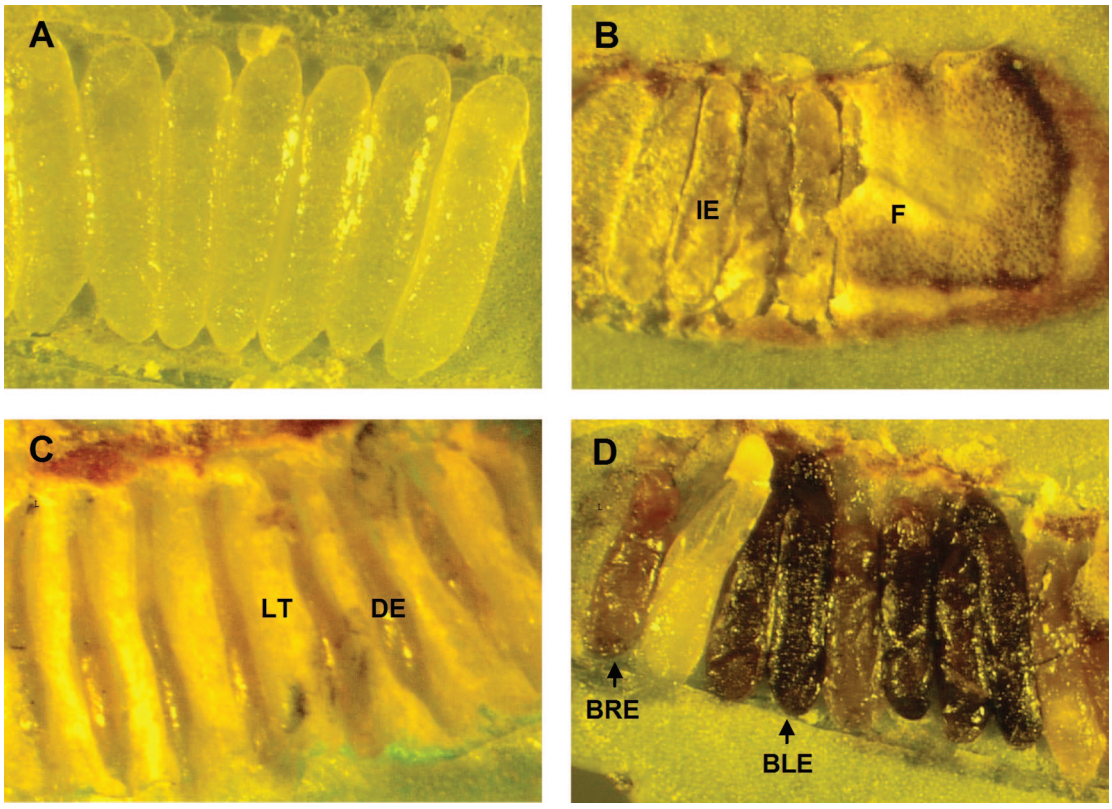

Fig. 1. (A–D) Morphological appearance of *H. coagulata* eggs observed during cold storage. (A) Newly refrigerated egg mass. (B) Egg mass infected by fungus after 40-d storage. (C) Eggs deformed by growth of leaf tissue after 60-d storage. (D) Deteriorating eggs (DE) after 60-d storage. IE, infected egg; F, fungus; LT, leaf tissue; BLE, blackened eggs; and BRE, brown eggs.

parallel, situated in chambers beneath the plant epidermis, and separated by transparent membrane-like plant tissues. After 40 d of storage, a few egg masses (<5%) became infected with a fungus. These eggs were gray and were covered gray or white fibrous-looking mycelia (Fig. 1B). The fungus first accumulated on the surface of the eggs, and then gradually totally invaded the inside of the eggs. The infested egg

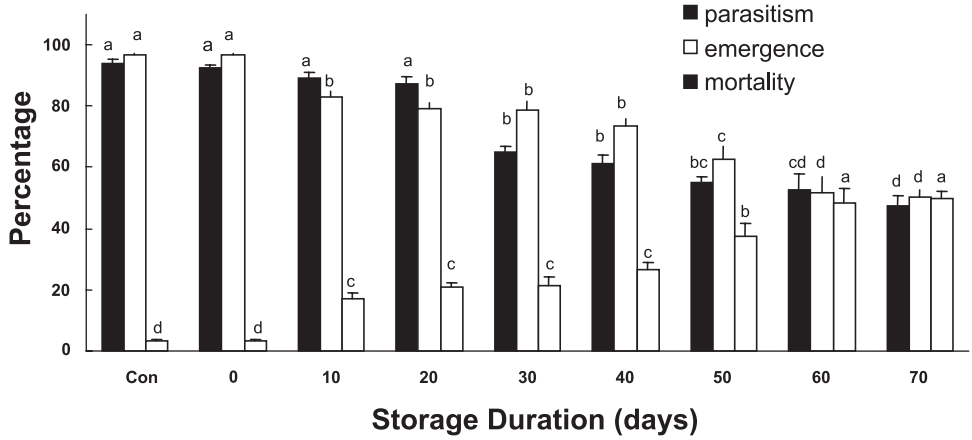

Fig. 2. Parasitism of previously refrigerated *H. coagulata* eggs by *G. ashmeadi* and subsequent offspring emergence and mortality. Each value represents means of five separate determinations and error bars represent ± 1 SE. Percentage of parasitism, emergence, and mortality were arcsine transformed to meet the assumption of normality before applying one-way ANOVA. Bars followed by the same letters are not significantly different at $\alpha = 0.05$.

masses were mostly rejected by the wasps; therefore, we eliminated them from the studies. The third category consisted of eggs damaged by growth of the host plant. Approximately 5–10% of the cuttings exhibited growth of plant tissues between the chambers after ≥60 d of storage, and this growth resulted in deformation and often rupture of the glassy-winged sharpshooter eggs (Fig. 1C). Also, after 60 d storage, some eggs (>10%) turned brown or black (Fig. 1D). These eggs were smaller than newly refrigerated eggs, probably due to water loss. When exposed to *G. ashmeadi* for 1 d, some of brown eggs were found to be parasitized, but no parasitoids were noted in black eggs.

Parasitism by P_1 Females and Emergence, Mortality, Sex Ratio, and Development of the F_1 Generation. Parasitism of refrigerated host eggs varied significantly with storage duration ($F = 59.25$; df = 8, 36; $P < 0.0001$), with the unstored, chill-terminated eggs having the highest levels of parasitism (Fig. 2).

Length of storage of the hosts significantly affected the emergence ($F = 54.98$; df = 8, 36; $P < 0.0001$), and mortality ($F = 32.98$; df = 8, 36; $P < 0.0001$) of the F_1 generation (Fig. 2). Parasitized, unstored eggs had an emergence rate of ≈46% more than those stored for 60 and 70 d. There was a tendency for a decline in emergence as storage duration increased.

The length of storage also affected the emergence patterns of the F_1 generation (Fig. 3A). Adult emergence persisted for 4 d for host eggs that were stored for 0 and 10 d, 7 d for those stored for 20 and 30 d, 8 d for those stored for 40 d, and 10 d for those stored for 50, 60, and 70 d, respectively. Peak emergence occurred on the first day of emergence for host eggs stored from 0 to 30 d, and on the second day for 40 d. There were two peaks of emergence for eggs stored for 50 through 70 d. The first peak emergence occurred on the third day of the emergence period, and the second peak occurred on the sixth day for those stored for 50 and 60 d and on fifth day for 70 d of storage. More than 78% of the progeny emerged within the first 2 d after the host eggs that were stored for 0–30 d, 43% for 40 d, 29% for 70 d, and <17% for 50 and 60 d.

Dissection of the previously stored hosts from which no emergence was noted showed that mortality of the progeny occurred at all stages during immature development. Within the eggs that were stored from 0 to 50 d, >89% of progeny death occurred during the pupal stage. For eggs that were stored for 60 d, the mortality of parasitoid progeny that occurred at the egg, larval, and pupal stages was 32, 5, and 63%, respectively; and for 70 d, 30, 13, and 57%, respectively. Overall, the death of parasitoid progeny occurring during development significantly increased as the length of storage increased (Fig. 2).

The developmental time of male, female, and the combined male and female progeny within the refrigerated host eggs varied significantly with storage duration (Table 1). However, the developmental time did not vary with the sex of progeny ($F = 1.58$; df = 1, 72; $P = 0.21$), and there was no interaction between storage duration and gender ($F = 1.00$; df = 8, 72; $P = 0.45$).

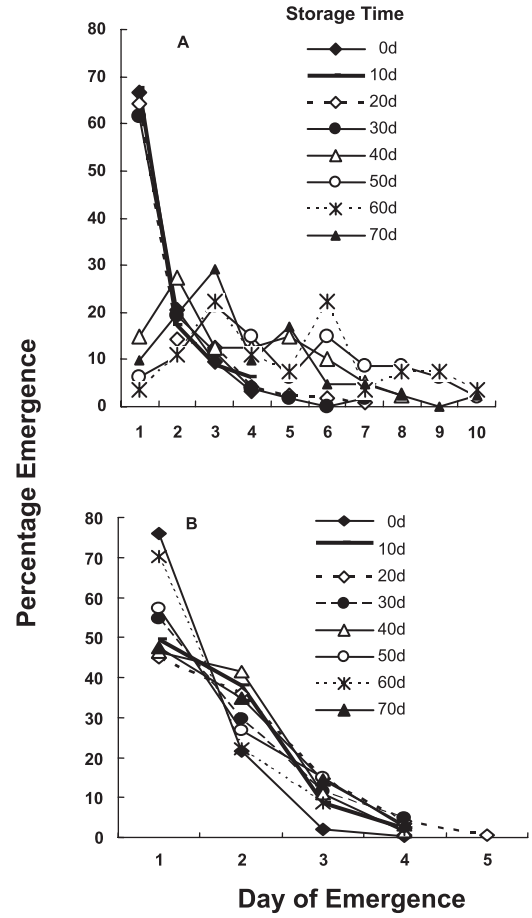

Fig. 3. (A and B) Adult emergence pattern of the F_1 (A) and F_2 (B) generations of *G. ashmeadi* reared on previously refrigerated *H. coagulata* eggs as a function of the storage time of the refrigerated host eggs. Each point equals the means of five and 10 separate replicates for F_1 and F_2 generations, respectively. To avoid confusion, the SE not included. For any given point, the value of the SE is <10% of the point value.

The number of male progeny emerging as adults significantly increased as the length of storage of the host eggs increased (Table 1). The male production from host eggs stored for 50, 60, and 70 d was 61, 69, and 80% higher, respectively, than that of eggs parasitized immediately after termination of development by incubation at 2°C for 5 d. However, untreated eggs (<24 h old) and unstored, terminated eggs produced the same proportions of male to female progeny.

Parasitism by F_1 Females and Emergence, Mortality, Development, and Sex Ratio of the F_2 Generation. When provided recently oviposited host eggs (<24 h old), the incidence of parasitism by the F_1 females from *G. ashmeadi* parents reared on host eggs previously refrigerated for 60 or 70 d varied significantly (Table 2).

Table 1. Developmental time of F_1 *G. ashmeadi* within *H. coagulata* eggs and the F_1 sex ratio as a function of length of previous cold storage

Storage duration (d)	Developmental time (d)			Sex ratio (% male offspring)
	Female	Male	Overall	
0	13.43 ± 0.32a	13.40 ± 0.16a	13.41 ± 0.17a	19.16 ± 1.52a
10	13.36 ± 0.27a	13.58 ± 0.13ab	13.47 ± 0.15a	21.61 ± 1.17a
20	13.72 ± 0.52a	13.76 ± 0.19b	13.74 ± 0.26a	23.49 ± 1.40ab
30	13.46 ± 0.22a	13.70 ± 0.13b	13.58 ± 0.13a	23.12 ± 2.23ab
40	15.06 ± 0.49b	14.06 ± 0.20b	14.56 ± 0.30b	28.57 ± 3.08bc
50	16.16 ± 0.54bc	16.01 ± 0.31c	16.13 ± 0.29c	30.88 ± 2.15c
60	16.84 ± 0.53c	15.84 ± 0.28c	16.34 ± 0.33c	32.42 ± 2.31c
70	16.28 ± 0.49c	16.20 ± 0.22c	16.24 ± 0.25c	34.46 ± 2.31c
Control	13.32 ± 0.32a	13.20 ± 0.06a	13.26 ± 0.16a	19.14 ± 2.04a
F	11.67	38.64	31.96	7.78
df	8, 36	8, 36	8, 72	8, 36
P	<0.0001	<0.0001	<0.0001	<0.0001

Ten refrigerated *H. coagulata* egg masses were exposed to *G. ashmeadi* colony for 2 d at 22 ± 1°C before they were placed in an experimental laboratory at 28 ± 2°C and a photoperiod of 24:0 (L:D) h. The experiment was repeated five times. Each value represents means ± SE of five separate determinations. Values of sex ratio were arcsine transformed to meet the assumption of normality before applying one-way ANOVA. Means in the same column followed by the same letter are not significantly different at $\alpha = 0.05$.

Emergence and the mortality of the F_2 *G. ashmeadi* occurring during development within the recently oviposited host eggs did not vary significantly with the length of previous storage of the hosts of parents (Table 2). The F_2 parasitoids of all groups also had a similar emergence patterns regardless of storage time (Fig. 3B). Adult emergence lasted 4–5 d, with peak emergence occurring on the first day of the emergence period. Furthermore, >81% of progeny emerged within the first 2 d.

Less than 2 and 4% and 12% of F_2 parasitoids died at the egg, larval, and pupal stages, respectively. Furthermore, the mortality of each of immature stages did not significantly vary with storage of the grandparents' hosts (Table 2).

Developmental time for females ($F = 0.84$; df = 7, 72; $P = 0.56$), males ($F = 1.51$; df = 7, 72; $P = 0.18$), and the combined female and male ($F = 1.81$; df = 7, 152; $P = 0.09$) of the F_2 generation within untreated *H. coagulata* eggs (< 24 h old) did not vary significantly with the length of storage of the hosts of the grandparents (Table 3). The developmental time of both sexes of the F_2 parasitoids was not significantly different ($F = 0.04$; df = 1, 144; $P = 0.84$). Moreover, there is no interaction between storage duration of the grandparents' hosts and the sex of the F_2 generation ($F = 0.61$; df = 7, 144; $P = 0.75$).

The sex ratio (female/male) of F_2 generation was strongly female biased. However, the proportion of males produced was not significantly affected by the storage duration of their grandparents' hosts ($F = 0.25$; df = 7, 72; $P = 0.97$), ranging from 19.5 to 24.6% (Table 3).

Longevity and Fecundity of F_1 Generation. Female longevity varied significantly with the storage duration of host eggs of the parent generation ($F = 8.97$; df = 7, 105; $P < 0.0001$) (Table 3). For those F_1 females emerging from host eggs that were unstored, but were chill terminated, longevity was ≈64% longer than those stored for 60 and 70 d, and ≈40% longer than those stored for 40 and 50 d.

Mated females of the F_1 generation oviposited into newly deposited host eggs on the first day of adult emergence. Their lifetime ($F = 8.82$; df = 7, 105; $P < 0.0001$) and daily fecundity ($F = 2.95$; df = 7, 105; $P =$

Table 2. Parasitism by F_1 *G. ashmeadi* reared on previously refrigerated *H. coagulata* eggs and the subsequent F_2 emergence and mortality as a function of length of storage

Storage duration (d)	Parasitism (% ± SE)	Emergence (% ± SE)	Mortality (% ± SE)			
			Egg	Larva	Pupa	Egg to adult
0	46.25 ± 2.06a	88.45 ± 1.46	0.89 ± 0.45	2.19 ± 0.75	8.47 ± 1.30	11.55 ± 1.46
10	43.38 ± 2.68a	87.80 ± 2.65	0.85 ± 0.57	2.42 ± 0.92	8.93 ± 2.12	12.20 ± 2.65
20	41.00 ± 2.33a	86.90 ± 2.27	0.92 ± 0.62	2.65 ± 1.44	9.53 ± 1.19	13.10 ± 2.27
30	46.00 ± 2.85a	87.15 ± 1.83	0.56 ± 0.38	3.31 ± 1.35	8.98 ± 2.16	12.85 ± 1.83
40	39.50 ± 2.84ab	86.15 ± 3.75	2.02 ± 1.46	2.00 ± 1.45	9.84 ± 2.23	13.85 ± 3.75
50	39.38 ± 4.39ab	86.34 ± 4.22	0.87 ± 0.62	2.71 ± 1.13	10.07 ± 3.48	13.66 ± 4.22
60	32.25 ± 2.08bc	87.18 ± 2.34	0.00 ± 0.00	1.79 ± 0.75	11.03 ± 2.19	12.82 ± 2.34
70	29.26 ± 1.97c	85.35 ± 3.78	1.59 ± 1.16	2.55 ± 1.43	11.52 ± 2.25	14.65 ± 3.78
F	4.99	0.10	0.61	0.16	0.15	0.11
df	7, 72	7, 72	7, 72	7, 72	7, 72	7, 72
P	<0.0001	0.9979	0.7430	0.9922	0.9830	0.9975

Storage time relates to storage of the hosts of the F_1 generation. Each value represents means ± SE of 10 separate determinations. Values were arcsine transformed to meet the assumption of normality before applying one-way ANOVA. Means in the same column followed by the same letter are not significantly different at $\alpha = 0.05$.

Table 3. F_1 longevity and fecundity of *G. ashmeadi* females reared on *H. coagulata* eggs after 0–70 d of cold storage and the developmental times and sex ratio of the F_2 progeny

Parameter	Storage time (d) at 10°C						
	0	10	20	30	40	50	60	70
Female adult longevity (d)	7.79 ± 0.82a	6.90 ± 0.86ab	6.80 ± 0.66ab	5.54 ± 0.76bc	4.47 ± 0.46c	4.70 ± 0.40c	2.83 ± 0.49d	2.67 ± 0.41d
Lifetime fecundity (e)	71.43 ± 4.85a	70.90 ± 6.77a	67.00 ± 5.39a	59.69 ± 5.79a	56.90 ± 3.94a	54.40 ± 4.17a	34.83 ± 2.54b	33.67 ± 3.23b
Daily fecundity (e)	10.21 ± 0.76b	10.69 ± 0.59b	10.16 ± 0.67b	11.39 ± 1.14b	13.20 ± 0.76ab	12.89 ± 0.77ab	14.78 ± 1.87a	14.83 ± 1.59a
Female developmental time (d)[a]	11.09 ± 0.20	11.53 ± 0.18	11.64 ± 0.25	11.53 ± 0.30	11.60 ± 0.18	11.35 ± 0.17	11.22 ± 0.24	11.38 ± 0.12
Male developmental time (d)[a]	11.02 ± 0.20	11.25 ± 0.12	11.65 ± 0.26	11.87 ± 0.33	11.27 ± 0.23	11.55 ± 0.21	11.31 ± 0.25	11.61 ± 0.09
Overall developmental time (d)	11.06 ± 0.14	11.39 ± 0.11	11.65 ± 0.17	11.70 ± 0.22	11.44 ± 0.15	11.44 ± 0.13	11.26 ± 0.17	11.49 ± 0.08
Sex ratio (% male offspring)	21.42 ± 2.78	23.99 ± 5.42	19.50 ± 2.04	23.24 ± 4.28	22.55 ± 3.91	24.57 ± 8.65	23.08 ± 3.57	22.17 ± 3.63

[a] Each value represents mean ± SE of 10 separate determinations, and each determination represents average developmental time (from oviposition to adult emergence) of emerged progeny that was produced by one parasitoid after 80 fresh host eggs were provided for 1 d. Means in the same row followed by the same letter are not significantly different at $\alpha = 0.05$. (d), data presented as days; (e), data presented as number of eggs per female.

0.0075) varied significantly with duration of storage of the host eggs provided for the parent generation (Table 3). Lifetime fecundity declined slowly with storage times ranging from 0 to 50 d, but no significant differences were detected. For F_1 females emerging from host eggs that were stored for 60 and 70 d, fecundity decreased by 51 and 53%, respectively, compared with egg-laying females emerging from chill-terminated, unstored eggs. Daily fecundity increased slowly with storage duration.

Demographic Growth Parameters for the F_1 Generation. Partial life tables were constructed for the number of *G. ashmeadi* adults entering each age class and for determination of the realized and actual mortality rate. Significant differences were detected among the demographic parameter estimates generated from jackknifed $l_x m_x$ data for the progeny from *G. asheamdi* reared on stored host eggs. The intrinsic rate of increase (r_m), net reproductive rate (R_0), mean generation time (T_c), population doubling time (T_d) and finite rate of increase (λ) were estimated by a jackknife analysis of $l_x m_x$ life table data at the different storage durations (Table 4). These demographic growth parameters were significantly affected by storage duration of the refrigerated host eggs. The parasitoids emerging from the refrigerated eggs that were stored from 0 to 50 d had the similar r_m, T_c, and λ values. Net reproductive rates decreased by ≥55% for the F_1 generation reared on host eggs that were stored for 60 and 70 d compared with those that were not stored. However, there was only 0.3- and 1.3-d difference in population doubling time and mean generation time across the different storage durations, respectively.

Discussion

This study documents the limits of refrigerated storage on the suitability of *H. coagulata* eggs for rearing *G. ashmeadi* and the fitness of the offspring. Examining the reproductive and developmental biology of the progeny is of vital importance when evaluating the utility of refrigerated host eggs for propagation of *G. ashmeadi* for release in a *H. coagulata* biological control program. Our results showed that eggs killed by 5 d of 2°C chilling and stored for 30 d at 10°C are suitable as hosts for rearing this parasitoid. It yields ≈70% parasitism and 80% emergence of adults from parasitized host eggs. Reproductive and developmental parameters further show that their progeny remain at a high quality without apparent damaging side effects. Storage for >40 d resulted in significant reductions in the incidence of parasitism and subsequent emergence rates. These results are consistent with a previous study (Leopold and Chen 2005).

The decline in parasitism by *G. ashmeadi* of glassy-winged sharpshooter eggs held for increasing periods in cold storage could have been caused by egg deterioration due to pathogen infection, structural damage, desiccation, or some combination of these factors. Fungal growth was evident on glassy-winged sharpshooter eggs stored for ≥40 d, with ≈10% of eggs

Table 4. Jackknife estimates of demographic growth parameters (± SE) for *G. ashmeadi* population reared on *H. coagulata* eggs stored for 0–70 d

Storage duration (d)	n	R_0	T_c	r_m	λ	T_d
0	14	51.47a (±3.55)	11.98a (±0.22)	0.32a (±0.00)	1.39a (±0.00)	2.11a (±0.02)
10	10	51.83a (±4.97)	12.08a (±0.21)	0.33a (±0.01)	1.39a (±0.01)	2.12a (±0.03)
20	10	44.16ab (±5.06)	12.27a (±0.24)	0.31a (±0.01)	1.36a (±0.01)	2.25a (±0.06)
30	13	37.75b (±4.55)	12.25a (±0.39)	0.30a (±0.01)	1.35ab (±0.01)	2.24a (±0.06)
40	15	33.65b (±3.01)	11.62a (±0.24)	0.30ab (±0.01)	1.35ab (±0.01)	2.26ab (±0.04)
50	20	38.90b (±3.06)	11.78a (±0.13)	0.30ab (±0.00)	1.36ab (±0.01)	2.24ab (±0.03)
60	12	22.37c (±1.80)	10.97b (±0.13)	0.28b (±0.01)	1.33b (±0.01)	2.45b (±0.06)
70	12	23.04c (±2.61)	11.26ab (±0.08)	0.28b (±0.01)	1.32b (±0.01)	2.49b (±0.08)

Means followed by the same letters within columns are not significantly different at $\alpha = 0.05$ according to a Student's t-test used to perform pairwise comparisons (two-tailed) of parameter estimates (Maia et al. 2000). n, number of females in analysis; growth parameters are described in text.

affected after storage for 70 d. Kanga et al. (2004) identified one fungal pathogen and two saprophytic fungi affecting *H. coagulata* nymphs and adults. The fungi affecting glassy-winged sharpshooter in our study were not identified. If fungi contribute consistently to the decline in glassy-winged sharpshooter egg quality after or during storage, fungicide applications could be used to minimize this problem.

Another kind of external damage occurring during storage was caused by growth of the leaf tissues between egg chambers of glassy-winged sharpshooter egg masses that severely deformed or burst the eggs. This mechanical type of injury resulted in a loss of ≈5–10% of eggs and was only observed as the duration of storage increased to ≥ 60 d. This type of injury could be reduced by administering a plant growth inhibitor to the cuttings on which the glassy-winged sharpshooter eggs are stored.

Some arthropod eggs that die from cold or chilling injury have been reported to turn black (Shintani and Ishikawa 1999). It is uncertain whether similar chilling injury caused the color change of the stored glassy-winged sharpshooter eggs. In this study, we did not find parasitoid eggs in the black host eggs even though they were exposed to a high density of wasps. In a few of brown host eggs, parasitoid eggs and first instars wasps were found. Although the brown eggs are able to trigger oviposition of *G. ashmeadi*, they were unable to support the continued development of the parasitoid. Nevertheless, it is evident that with lengthening storage, the browning and blackening of the host eggs caused a portion of the decline in parasitism and emergence rates.

For F_1 generation, the biological parameters, including parasitism, emergence, emergence patterns, mortality, and development, were dependent on the length of storage of refrigerated host eggs (Table 1; Fig. 2). When host eggs were stored up to 30 d, these parameters, except the emergence rate, were similar. Thus, the only manifested decline in host quality up to 30 d of storage was limited to the 19% reduction in the emergence rate. For F_2 generation reared on untreated host eggs, their emergence rate and pattern, mortality, and development were similar (Table 2; Fig. 2). This indicates 1) that these parameters of F_2 generation are not influenced by the length of storage of refrigerated host eggs that were provided for grand parental generation and 2) that the latent detrimental affect of cold storage of the hosts that was evident during the F_1 generation does not impact the F_2 generation when offered fresh eggs.

The longer storage times could have resulted in a decrease in the nutritive value of the egg contents and the subsequent delay in development. Some of host eggs (e.g., brown eggs) after 60 d of storage were parasitized, but they did not support progeny development past the first instar. The numbers of parasitoids dying at the egg stage increased to 30% after storage for ≥60 d. Meanwhile, nearly 90% of the undeveloped progeny in the uncolored host eggs died at the pupal stage within hosts stored for ≤50 d. These results indicate that when the host eggs were stored longer they were less suitable for supporting parasitoid development. When previously making dissections of parasitized untreated host eggs, we noted that death of the *G. ashmeadi* embryos before hatching was an extremely rare event (Chen et al. 2006b). The decline in the suitability of host eggs stored >30 d for supporting parasitoid development was also accompanied with a significant extension of the parasitoid developmental time and was expressed in the form of different emergence patterns.

The F_1 and F_2 generations retained a female-biased sex ratio across the different storage times. This result is in accord with the studies conducted by Pilkington and Hoddle (2006) and Chen et al. (2006b). However, we found that the proportion of male progeny in the F_1 generation significantly increased as storage duration increased, whereas that in F_2 generation remained similar to control levels. This increase may be related to the change in size of host eggs during storage. Our microscopic observations indicated that the host eggs became smaller after storage for ≥40 d. The host size models for solitary parasitoids predict that the females oviposit a greater proportion of their daughters in the larger hosts and sons in the smaller hosts when encountering both host sizes (Charnov 1979, Charnov et al. 1981). Thus, shrinkage of host eggs, most likely caused by desiccation with the lengthening of the storage times, may account for the increase in the number of male progeny.

The effects of extended refrigerated storage of host eggs on the quality of the F_1 generation were evident. The fitness costs to those progeny emerging from host eggs after 30-d storage were significant, and after 50 d, they were substantial when based on demographic parameters. For the F_1 females, the longevity decreased as the storage duration increased and the shortened longevity resulted in a decrease in fecundity. When the other parameters are assessed, the total impact of refrigerated host storage on the quality of F_1 generation can be visualized. The R_0 value decreased after storage for 30 d, and by 60 d this value had decreased significantly. However, it was only after $\geq$60 d that the F_1 generation exhibited the severe reductions in r_m and λ and the related increases in T_c and T_d. It also should be noted that there was only 0.3 and 1.3 d difference across the various storage times for T_d and T_c values, respectively, and these differences are not of biologically significance when considering the 1-d time period allowed for oviposition and 1-d interval between observations. The parameters r_m, λ, T_c, and T_d for the F_1 generation from host eggs stored for $\leq$50 d were the same as that of *G. ashmeadi* reared on fresh eggs in previous studies (Chen et al. 2006b, Pilkington and Hoddle 2006).

In summary, our study indicates that refrigerated storage at 10°C of chill-terminated eggs of *H. coagulata* stored for up to 30 d can be used as the hosts for rearing *G. ashmeadi* without damaging side effects on population growth parameters. Furthermore, although storage of host eggs from 30 to 50 d has various effects on developmental fitness of the F_1 generation, it does not severely affect the reproductive fitness. All net reproductive rates across the various storage times have values equaling >20, indicating that the F_1 progeny are able to multiply rapidly when normal host eggs are available. Typically, glassy-winged sharpshooter egg production declines considerably in the winter season (from mid-November to mid-January) in the field and under mass-rearing conditions due to a reproductive diapause (Hummel et al. 2006). In this case, the use of refrigerated storage for saving host eggs up to 50 d for later use can be practical method for maintaining *G. ashmeadi* colonies. Improvement of the suitability of host eggs for wasp propagation by delaying leaf tissue growth and avoiding fungal infestation and egg deterioration are among the features that would make refrigerated storage even more useful.

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